BIODESULFURIZATION SYSTEMS FOR REMOVAL OF ORGANIC SULFUR FROM COAL: A CRITICAL REVIEW

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ABSTRACT

This study evaluates approaches for the biological removal of organic sulfur from coal. In this area, Atlantic Research Corporation's (ARCTECH's) biodesulfurization system is the only one that has been demonstrated on coal with mixed success on a continuous bench-scale unit of 10 lb/day capacity. Other biocatalytic systems developed by the Institute of Gas Technology (IGT) or Southern Illinois University (SIU) are still in the laboratory-scale microbial selection and screening stage. Yet, the successful use by IGT of a sulfur-limited, continuous chemostat for the selection of bacterial strains with appropriate desulfurization activities has provided a convenient and powerful strain selection technique. The IGT work has also established the possibility of changing the metabolic pathway by proper modification of the bacterial growth medium and introduced the sulfur bioassay technique to help compare the effectiveness of different microorganisms grown with different substrates under different conditions on a comparable basis. SIU was successful in mapping the desulfurization genes in a mutant *E. coli*.

There is still a need for faster growing, stable, and more active biodesulfurization microorganisms than those which have been developed so far. This calls for a program of strain selection and improvements through molecular genetics, a thorough understanding of coal biodesulfurization metabolism and its associated metabolic pathways, investigation of extracellular enzymatic removal of organic sulfur from coal, and identification of new acidophillic heterotrophs that have broad organic sulfur removal capabilities and that can coexist with other bacterial strains currently used for inorganic sulfur removal.

BACKGROUND

Historically, most of the research on the biological processing of coal was directed to pyrite removal; very few studies have been explicitly devoted to organic sulfur removal although there are three studies that are particularly interesting. One study conducted in 1979 by Chandra et al.[1] indicated that a heterotrophic bacterium, enriched on dibenzothiophene (DBT), can remove up to 20 percent of the organic sulfur present in Indian coal after 10 days of incubation in a laboratory rotary shaker at 30°C. Another study conducted by Gokcay and Yurteri[2] in 1983 on Turkish lignite showed that 50 to 57 percent of the organic sulfur and 90 to 95 percent of the pyritic sulfur were removed over a 25-day incubation period. The third study was conducted by Kargi and Robinson[3] at Lehigh University using bituminous coal suspended in a growth medium inoculated with *Sulfolobus acidocaldarius* organism. After 28 days of incubation with the DBT-adapted culture at 70°C, nearly 19 percent of the initial organic sulfur in the pretreated coal was removed. Further studies on the ability of the *Solfolobus* species to desulfurize coal continued at Lehigh University in 1983 under the sponsorship of the U.S. Department of Energy's (DOE) Pittsburgh Energy Technology Center (PETC).

CURRENT STATE OF THE ART

Since 1983, DOE has continued to sponsor the investigation of various biological approaches for organic sulfur removal from coal at various U.S. institutions. Thus, ARCTECH Inc. (formerly Atlantic Research Corporation) was funded to continue their work on the development of *Pseudomonas* and other "Coal Bugs" that were able to release organically-bound sulfur from DBT and from selected coals. The Institute of Gas Technology (IGT) was funded to investigate the general feasibility of the microbial removal of organic sulfur from coal. Southern Illinois University (SIU) was also funded under DOE's University Coal Research Program in support of the overall coal biodesulfurization mission. All these DOE-supported research efforts are critically reviewed in this report.

ARCTECH Inc. has isolated a mutant *Pseudomonas* microorganism designated CB1 ("coal bug 1") that has shown the ability to remove sulfur, both from model sulfur compounds and from various coals.[4] Studies using dibenzothiophene (DBT) indicate that CB1 appears to be most effective in removing thiophenic sulfur. In laboratory-scale studies of coal desulfurization, CB1 reduced the percent organic sulfur by 18 and 47 percent at residence times of 9 - 18 hours depending on the coal, particle size, initial organic sulfur content and other, as yet unidentified, parameters. Various coals have also been treated with CB1 in a continuous bench-scale unit that can process 10 pounds/day of coal. Percent organic sulfur reductions varied from 10 to 29 weight percent, depending apparently on the coal and other unidentified parameters.

ARCTECH Inc. has also isolated another microorganism designated CB2 that has shown the ability to oxidize aryl sulfide model compounds like diphenyl sulfide (DPS) and benzyl phenyl sulfide (BPS). This microorganism was also tested on various coals with mixed success. Between 14 and 34 weight percent organic sulfur reduction was achieved for three coals. Again, the variation in effectiveness seemed to be coal dependent, but other factors were obviously present. Like CB1, CB2 has a negligible effect on the pyritic sulfur.[4]

Since experimental evidence from model compound studies indicated that CB1 and CB2 were metabolizing different sulfur functionalities, coal desulfurization experiments were performed by ARCTECH using a mixed culture of CB1 and CB2 in an attempt to improve the overall sulfur removal. The results indicated that coal desulfurization using mixed cultures was generally less effective than that achieved using the pure culture.[4] Furthermore, it appears that a metabolic by-product of CB2 inhibits the growth of CB1, thus allowing CB2 to become predominant in the total biomass.[4] This indicates that sequential desulfurization using the two cultures independently may be the preferred and only solution.

The Institute of Gas Technology has developed a sulfur bioavailability assay to identify microorganisms capable of degrading model sulfur compounds.[5] Recently (1989) efforts in this area have resulted in the successful adaptation of the IGT Sulfur Bioavailability Assay to microtiter plates.[6] This allows large numbers of mutagenized colonies to be conveniently screened to detect desulfurization-deficient mutations. Using this bioassay, IGT has identified a strain of microorganism designated IGTS7 that, when grown on several carbon substrates, is capable of degrading a wide variety of model sulfur compounds, including DBT. Using a sulfur-limited continuous chemostat, IGT further tested this microorganism on Illinois #6 coal. The chemostat effluent was monitored for the presence of metabolizable sulfur, and two peaks were found corresponding to 30 and 70 days of operation. It was surmised that the second peak represented the metabolism of organic sulfur in the coal by the IGTS7 strain that had survived the continuous operation of the chemostat during the sulfur-free period following the decline of the first peak. To test this, samples of coal were analyzed initially, at day 53 and at day 91. The last sample indicated a decrease in organic sulfur content for the coal of about 24 weight percent. Subsequent to this work, IGT has reported an organic sulfur removal of 90 percent using this microorganism after 212 days of chemostat operations. The coal apparently experienced a carbon loss of 39 percent during this procedure. Such an extraordinarily high sulfur removal needs to be replicated in further experiments at considerably shorter residence times before general scientific acceptance is forthcoming. The major thrust of current experimental efforts at IGT is the isolation of pure cultures out of the IGTS7 mixed culture.[7] A pure culture capable of desulfurization would greatly aid future research in genetics. Two pure cultures of bacteria

that are each capable of utilizing dibenzothiophene (DBT) as their sole source of sulfur were isolated from the mixed IGTS7. These cultures have been identified as *Rhodococcus rodochroas* and *Bacillus sphaericus* species, and have been designated IGTS8 and IGTS9, respectively.[7] None of these cultures alone was found capable of sulfur-specific metabolism. However, the pairwise combinations of any of these cultures with *Enterobacter* species can reproducibly perform well in the Sulfur Bioavailability Assay. At this point, it is believed that the *Enterobacter* species is only a nutritional component needed for the growth of the *Rhodococcus* and *Bacillus* species, with no contribution to desulfurization.[8]

Research at SIU at Carbondale has utilized two approaches for isolating and developing bacteria capable of removing organic sulfur from coal: enrichment culture and genetic manipulation. In the enrichment culture approach, which incidentally is the approach used by ARCTECH, the organism is isolated from naturally-occurring bacteria and adapted for growth on model sulfur compounds. The adapted organism is then subjected to a repetitive selection and mutation cycle to provide the enriched culture with the desired traits. The desulfurization potential of the isolated strains was determined by measuring sulfate and/or hydrogen sulfide released during bacterial degradation of model sulfur compounds. Several isolates were selected that could degrade dibenzothiophene sulfane (DBTS), dibenzothiophene (DBT), benzene sulfonic acid (BSA) and cystine (CYI).[9] The isolates that degraded the latter compound were particularly active in their growth and were selected as potential candidates for future coal desulfurization studies.

The genetic manipulation approach of SIU involves mutation of *E. coli*, a genetically well-understood organism for metabolizing sulfur containing amino acids. *E. coli* NAR3 is a bacterial strain produced after successive cycles of mutation and selection. This strain can degrade thiophenes, furans, and other sulfur-containing aromatic compounds.[10] Genetic analysis of NAR3 has been undertaken at SIU, and since then other mutants showing improved degradation of thiophene and other sulfur containing aromatic substrates have been isolated. One of these, designated NAR41, has shown increased affinity for thiophene rings and decreased affinity for other non-sulfur containing rings.[10] This is clearly in the right direction since the goal is to remove sulfur with as little loss of coal carbon as possible.

CRITICAL REVIEW

The basic goal of all biodesulfurization processes is to remove the organically-bound sulfur from coal while retaining the fuel value of the coal. This means that biodesulfurization should follow a metabolic pathway that eliminates sulfur with little destruction of the coal carbon. Investigations to date have focused on the metabolic pathway analysis for DBT and not for coal. In the desirable pathway, the so-called 4-S, the DBT is successively oxidized to sulfate and 2,2'-dihydroxybiphenyl.[11,12] The other pathway results in destruction of the aromatic ring structure of DBT with no liberation of sulfur. CB1 apparently releases sulfur from DBT as sulfate, and 2,2'-dihydroxybiphenol has been identified as the organic product. Thus, CB1 seems to operate via the 4-S pathway. IGT finds monohydroxybiphenyl as a product, so it is likely that IGTS7 metabolizes DBT using a variant of the 4-S pathway. The other microorganisms under development appear to involve some participation of carbon-destructive metabolic pathways.

All coal desulfurization organisms developed so far have been recovered from microbial populations isolated from soil near coal mines or petroleum refineries. These microorganisms are single cell (procaryotes), rod-like aerobic bacteria that remain active only in neutral or alkaline medium at temperatures between 25 and 35°C. Because of this, they are unable to coexist with those acidophillic heterotrophs currently used for inorganic sulfur removal. Furthermore, the metabolism of most of these organisms is poorly understood at present, and therefore, their growth media have generally not been optimized.

Despite its attractive potential, the biodesulfurization of coal has its problems and limitations. One major problem is the heterogeneity of coal, which means that the same microorganism may not be effectively used with all types of coal. In other words, the biological removal of organic sulfur from coal may have to be tailored to each coal separately. Possible instability of genetically-engineered microbial cultures is another problem. Over

extended periods of usage, an originally effective desulfurization organism may give rise to spontaneous derivatives that lack desulfurization ability. The long residence time required for bioprocessing is also a major obstacle to the usefulness of this technology. Low biodesulfurization rate, slow bacterial growth, and low process yield may all contribute to limit future application of biodesulfurization. The other potential limiter is the surface availability of the organic sulfur for microbial attack.

For any desulfurization process to be viable from a utility boiler aspect, it should be capable of reducing the sulfur content of coal to produce compliance fuels. For typical U.S. coals with 3 percent total sulfur and assuming 90 percent physical removal of inorganic sulfur, the removal of 50 percent of the organic sulfur is required. Thus, about 60 percent of the total coal sulfur must be accessible for microbial metabolism at the coal surface. This can be achieved theoretically with finely-ground coal having an average particle size of 38 microns (200-400 mesh), assuming that the thickness of the outer coal surface layer accessible for microbial action is 5 microns. Fine grinding of coal can be both an economic and a technical penalty since dewatering of coal slimes is a difficult problem. Whether microbial action can penetrate below the coal surface for non-extracellular enzymatic processes remains to be investigated.

An important economic criterion is the growth rate of the microorganism. This determines the capacity of the fermenters and therefore impacts capital cost. Economically attractive growth rates of 0.66 $hour^{-1}$ have been quoted in the literature,[13] and this corresponds to a bacterial generation time of 1.05 hours, compared to 3.8 hours for CB1 and 4.0 hours for CB2. Other microorganisms isolated so far in the biodesulfurization program have much longer generation times of two days or more. Another consideration is the biomass yield. This determines the necessary growth media, oxygen demand, productivity, plant size and thus operating and capital costs. The typical economic biomass yield for *Pseudomona* grown on benzoic acid is 0.60 grams of dry cell biomass per gram of benzoic acid consumed. This is 15 percent higher than that achieved by CB1 under current growth conditions on benzoic acid. The kinetics of the process also determine economic viability. Assuming first-order biodesulfurization kinetics and removal of 50 percent organic sulfur at the best currently achieved residence time of 9 hours, the rate constant is 0.08 $hour^{-1}$. The desulfurization rate constant for CB1 currently ranges between 0.01 and 0.05, depending on conditions and the coal type.

CONCLUSIONS AND RECOMMENDATIONS

This analysis of the various techniques being investigated for the chemical cleaning of coals has not been able to positively identify the most promising approaches to this problem. One reason is lack of overall process data in much of the current research effort. Emphasis has been on overall sulfur removal efficiency. The relative proportion of organic vs inorganic sulfur has often not been identified. The issues of carbon losses and product characteristics have essentially been neglected. Other aspects generally not addressed in current research are the potential costs and process energy requirements.

If, as conventional wisdom suggests, the organic sulfur in coal is an integral part of the coal matrix, then disruption of the coal matrix must occur before organic sulfur can be removed. This disruption implies that the product may have properties and characteristics different from the parent coal, this difference being a function of the severity of the desulfurization process.

These considerations point to major recommendations for research priorities in the area of coal desulfurization. Of prime importance is to attempt to develop analytical techniques for identifying organic sulfur speciation. Potential techniques are already in existence, and very likely several of them will have to be used in combination to unequivocally assign sulfur functionalities in the coal matrix. Once we know what compounds we are dealing with and have a reliable way to measure them, we stand a better chance of developing chemistry and biochemistry that will remove them.

In addition, research in coal desulfurization must constantly be concerned not only with desulfurization efficiency, but also with the characteristics and potential uses of the desulfurized product. Superclean coals may well be superclean from the standpoint of low sulfur and mineral matter content, but they may also have limited utility as a fuel form.

The use of biological means for the removal of organic sulfur from coal must be looked upon as a potential long-term development. Assuming that stable microorganisms can be developed that degrade organic coal sulfur, there are still many uncertainties yet to be resolved before biodesulfurization of coal becomes a realistic commercial option. These uncertainties include (1) a realistic estimate of the accessible organic sulfur available for microbial metabolism at the coal particle surface, (2) reliable determination of the various sulfur species in coal, (3) scale-up considerations for bioreactors, and (4) reasons for the variable response of different coal types to bioprocessing.

The ARCTECH biodesulfurization system is currently the only one that has been demonstrated on coal, although with mixed success, using a continuous bench-scale unit of 10 lb/day capacity. Other biocatalytic systems developed by IGT or SIU are still in the laboratory-scale microbial selection and screening stage. The research work of IGT and SIU has, nevertheless, contributed significantly to the coal biodesulfurization mission. IGT's successful use of a sulfur-limited, continuous chemostat for the selection of bacterial strains with appropriate desulfurization activities has provided a convenient and powerful strain selection technique. The IGT work has also established the possibility of changing the metabolic pathway by proper modification of the bacterial growth medium, and has introduced the sulfur bioassay technique to help compare on a common basis the effectiveness of different microorganisms grown with different substrates under different conditions. SIU was successful in mapping the desulfurization genes in a mutant *E. coli*. This is an important step towards the application of molecular genetics for the development of improved bacterial strains with enhanced desulfurization capabilities.

The conclusion is that there is a need for faster growing, stable, and more active biodesulfurization microorganisms than those that have been developed so far. To this end, there are several major research needs that can be identified. There is a need for a structured and systematic program of strain selection and improvements through molecular genetics, and a need for a thorough understanding of coal biodesulfurization metabolism and its associated metabolic pathways. A similar approach was successfully applied in penicillin production and resulted in a thousandfold increase in yields. There are also needs to investigate extracellular enzymatic removal of organic sulfur from coal, and to identify new acidophillic heterotrophs that have broad organic sulfur removal capabilities and that can coexist with other bacterial strains currently used for inorganic sulfur removal.

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